Microvascular dysfunction, physical activity, and cardiometabolic diseases

Summary

In **Chapter 1**, we have described the worldwide epidemic of cardiometabolic diseases, which leads to lower quality of life, additional healthcare expenditure, and risk of early death [1,2]. Investigations on the etiology can provide evidence for effective prevention and treatment. Microvascular dysfunction and physical (in)activity may play an important role in the development of cardiometabolic diseases, which, however, has not been well elucidated. Therefore, we aimed to investigate the association among microvascular dysfunction, physical (in)activity, and cardiometabolic diseases in a population-based study, The Maastricht Study [3]. In this dissertation, we applied advanced technologies which enabled more accurate and comprehensive assessments of microvascular function and physical behavior. The description of measurement protocols is necessary for researchers to identify whether differences in results across studies are based on differences in methodologies, especially for the measurements that are yet to be standardized. In **Chapter 2**, we described the protocols of the microvascular measurements applied in The Maastricht Study, including non-invasive measurements in skin, retina, brain, and sublingual tissue as well as plasma and urine biomarker assessments. The use of these measurements enables the study of microvascular changes in various (patho)physiological conditions, as well as their similarity and difference across the territories. Following this, we summarized the main findings in The Maastricht Study involving these microvascular measurements up to 2018. These cross-sectional studies have demonstrated associations between multiple cardiovascular risk factors (age, sex, blood pressure, waist circumference, etc.) and diseases (e.g. (pre)diabetes and depression) and microvascular (dys)function [4-13]. Finally, we provide a brief perspective of future microvascular investigations in The Maastricht Study, including fully automated analysis of microvascular imaging and longitudinal studies. We can already see some remarkable progress in the following chapters of this dissertation.

In **Chapter 3**, we investigated whether (pre)diabetes and plasma glucose levels were associated with retinal microvascular diameters. We performed a cross-sectional study in the framework of The Maastricht Study, including 2876 participants (n=1630 normal glucose metabolism, n=433 prediabetes, and n=813 type 2 diabetes). We used oral glucose tolerance test (OGTT) to define the glucose metabolism status. We took fundus photographs of each participant and used a semi-automated software (retinal health information and notification system [RHINO]) to determine retinal arteriolar and venular diameters. Multivariable regression analyses were performed and adjusted for age, sex, waist circumference, smoking status, systolic blood pressure, lipid profile, and the use of lipid-modifying and/or antihypertensive

medication. The results showed that type 2 diabetes, higher levels of hemoglobin A1c (HbA1c), and, possibly, prediabetes were significantly associated with wider retinal arteriolar diameters in a predominantly white population, independently of a broad array of potential confounders. The associations of (pre)diabetes and HbA1c with retinal venular diameters were directionally similar to those for arterioles, even though they were not statistically significant after adjustment for potential confounders. We additionally explored the association between retinal arteriolar and venular diameters, and found that retinal arteriolar diameters were associated with retinal venular diameters, independently of age, sex, height, body surface area, systolic blood pressure, and HbA1c level. Through more accurate assessments of the exposure and outcome, extensive adjustments for potential confounders, and the broad array of additional analyses, this study provided robust evidence and supported the concept that retinal microvascular changes already occur before the diagnosis of type 2 diabetes.

In **Chapter 4**, we explored the association of generalized microvascular dysfunction with beta cell function in 2275 participant without history of diabetes in The Maastricht Study. We used 7-point OGTT to assess the fasting insulin secretion and glucose-stimulated insulin secretion. We assessed microvascular function by plasma biomarkers of endothelial function, urinary albumin excretion, retinal microvascular diameters, flicker light-induced retinal microvascular dilation, heat-induced skin hyperemia and calculated a composite score. The results showed that higher plasma biomarkers, higher urinary albumin excretion, and wider retinal microvascular diameters were significantly associated with higher insulin secretion in the fasting state. Higher levels of plasma endothelial biomarkers and urinary albumin excretion were associated with higher glucose-stimulated insulin secretion in the late phase. Notably, these associations are independent of insulin sensitivity and other potential confounders. Our study is the first population-based study to show a close relationship between microvascular dysfunction and beta cell function in humans *in vivo*. The findings support the hypothesis that islet microvascular dysfunction may contribute to altered insulin secretion. In contrast, most of previous animal and *in vitro* studies have shown an attenuated glucose-stimulated insulin secretion induced by islet microvascular dysfunction. We may attribute the inconsistency to species differences. In addition, most of our study population had normal glucose tolerance.

In **Chapter 5**, we investigated whether systemic microvascular dysfunction was associated with incident cardiovascular disease, and if so, whether the associations differ across different vascular beds. Among 2531 participants in The Maastricht Study, we assessed microvascular function in multiple territories, including brain, retina, skin, plasma and kidney. A composite score of microvascular dysfunction was calculated. The follow-up of cardiovascular disease

was performed by use of an annual questionnaire. After a median follow-up of 5 years, we found that a higher composite score was associated with a higher risk of incident cardiovascular disease in the fully adjusted Cox regression model. In addition, the associations were not significantly different with regard to outcomes in coronary, cerebral, and peripheral arteries. Based on these findings, systemic microvascular dysfunction may play an important role in the development of cardiovascular diseases in different vascular beds.

In **Chapter 6**, we examined the association between the volume and the pattern of sedentary behavior and physical activity with incident cardiovascular disease. We included 4706 participants without history of cardiovascular disease (n=336 had incident events) in The Maastricht Study. We used the activPAL3 activity monitor to assess physical variables, including sedentary time, light-intensity physical activity (LIPA), moderate-to-vigorous-intensity physical activity (MVPA), vigorous-intensity physical activity (VPA), number of sedentary breaks, number of prolonged sedentary bouts ($\geqslant$30 minutes), average sedentary bout duration, and physical activity pattern. We performed a follow-up of cardiovascular disease using an annual questionnaire (median follow-up=5.1 years). The results showed a significant sex difference in the association of volume of sedentary behavior and physical activity with incident cardiovascular disease. In women, more MVPA was associated with an increased risk of developing cardiovascular disease. The association was not independent of mobility limitation and body mass index (BMI). In men, more LIPA was associated with a higher risk of cardiovascular disease, independently of potential confounders and mediators. This study is the first prospective study including middle to older aged population to investigate the association of accelerometer-measured sedentary behavior and physical activity with incident cardiovascular disease and show the shape of dose-response relations in sex subgroups. The linear association of MVPA with incident cardiovascular disease in women supports the advice on MVPA in the current guidelines, though meeting guidelines of >150 minutes/week of MVPA was not significantly associated with incident cardiovascular disease. In addition, the association of LIPA with incident cardiovascular disease in men may suggest a potential role of occupational physical activity in the development of cardiovascular disease.